

Study Title

Biodegradation of PES Vorstufe 2342

Data Requirements / Test Guidelines EU method C.4-D (2008) OECD TG 301 F (1992)

Author:

Prof. Dr. Caspers

Study completion date:

2010-07-20

Sponsor:

Bayer MaterialScience AG
BMS-IO-ST-PSRA-GPRA
51368 Leverkusen
Germany

Testing facility:

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Laboratory Project Identification

Study No. 2010/0087/14

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1. GLP DECLARATION

This study was conducted in compliance with the OECD principles of Good Laboratory Practice (1999) and with the Principles of Good Laboratory Practice according to Annex I, German Chemical Law (2008).

Date / Signature

Study Director

(Prof. Dr. Caspers / Neuhahn)

Study No.: 2010/0087/14

2. ARCHIVING

The original report, the study plan, and all raw data pertaining to this study are stored in the "GLP Archive, CURRENTA GmbH & Co. OHG, Services Analytik, CHEMPARK, Building Q 18, 51368 Leverkusen". A sample of the test item is stored in "GLP-Sample Store, CURRENTA GmbH & Co. OHG, Services Analytik, CHEMPARK, Building DA1, 41538 Dormagen".

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3. QUALITY ASSURANCE STATEMENT

This report was audited by the Quality Assurance Unit CURRENTA Services Analytik, Quality Management at CURRENTA GmbH & Co. OHG and this statement confirms that the final report reflects the raw data.

The dates of Quality Assurance inspections and audits are given below.

Audits	Dates of QAU inspections	Dates of reports
study plan review process based inspection	2010-06-08 2010-04-21 2008/0121/11	2010-06-08 2010-04-21
final report review	2010-07-23	2010-07-73

Date / Signature

2010-07-23 A-Securic (Senic /-Dr. Dörzbach-Lange / Dr. Neupert)

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4. STUDY TIME TABLE

Study initiation date: 2010-06-08

Study completion date: 2010-07-20

Start of experimental phase: 2010-06-08

End of experimental phase: 2010-07-20

5. **GLP CERTIFICATE**



Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz des Landes Nordrhein-Westfalen

nrift: 40190 Düsseldor

Aktenzeichen: VI-3-31.11.65.05

Study No.: 2010/0087/14

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance (gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der Assessment of conformity with GLP according to GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie Chemikaliengesetz and Directive 88/320/EEC at: 88/320/EEC at:

☑ Prüfeinrichtung/Test facility
☐ Prüfstandort/Test site Bayer Industry Services GmbH & Co OHG Prüfeinrichtung BIS-SUA-Analytics D-51368 Leverkusen

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)

(according ChemVwV-GLP Nr. 5.3/OECD guidance)

Kategorie 1

Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen

physical-chemical testing

Areas of Expertise

category 4

Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen

environmental toxicity studies on aquatic and terrestrial organisms

Kategorie 5

Prüfungen zum Verhalten im Boden, im Wasser und in studies on behaviour in water, soil and air; der Luft, Prüfungen zur Bioakkumulation und zur bioaccumulation Metabolisierung

Kategorie 8

category 8

Analytische Prüfungen an biologischen Materialien

analytical and clinical chemistry testing

Datum der Inspektion (Tag.Monat.Jahr)

Date of Inspection (day.month.year)

14. bis 16. September

on 14 until 16 September and on 26 until 28

und 26. bis 28. Oktober 2005

October 2005

Die genannte Prüfeinrichtung befindet sich im nationalen The above mentioned test facility is included in the national GLP-Überwachungsverfahren und wird regelmäßig auf GLP Compliance Programme and is inspected on a regular behalbung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit Based on the inspection report it can be confirmed, that this bestätigt, dass in dieser Prüferinrichtung die oben test facility is able to conduct the aforementioned studies in genamten Prüfungen unter Einhaltung der GLP- compliance with the Principles of GLP.

Düsseldorf, den AJanuar 2006 Im Auftrag

Dienstsiegel/official-seal

Please note: Effective January 1st, 2008 the company name Bayer Industry Services GmbH & Co. OHG was changed to CURRENTA GmbH & Co. OHG

6. SUMMARY

Study No.: 2010/0087/14

A study was performed to assess the ready biodegradability of PES Vorstufe 2342.

The study was conducted in accordance with the Council Regulation (EC) No 440/2008, Method C.4-D "Manometric Respirometry Test" (2008). This test method is in all essential parts identical with OECD Guideline 301 F (adopted July 1992).

A solution of PES Vorstufe 2342 in a mineral medium was inoculated and incubated for 28 d under aerobic conditions. During this period, degradation was followed by continuous automated BOD determinations.

PES Vorstufe 2342 showed:

- 37 % degradation after 7 days
- 54 % degradation after 14 days
- 67 % degradation after 21 days
- 72 % degradation after 28 days

As the degradation within the 10-d window was less than 60 % removal, PES Vorstufe 2342 is considered to be "Not Readily Biodegradable".

The reference compound sodium benzoate showed 79 % degradation after 14 days.

7. EXPERIMENTAL PROCEDURE

Study No.: 2010/0087/14

This study was designed to assess the ready biodegradability of PES Vorstufe 2342. It was conducted in accordance with the Council Regulation (EC) No 440/2008, Method C.4-D "Manometric Respirometry Test" (2008). This test method is in all essential parts identical with OECD Guideline 301 F (adopted July 1992).

A solution of 100 mg/L test item in a mineral medium, to give at least 50-100 mg COD/Litre as the nominal sole source of organic carbon, was stirred in a closed flask and inoculated at a constant temperature (22 \pm 1 °C) for up to 28 days under aerobic conditions in the dark.

The consumption of oxygen (BOD) was determined by measuring the drop in pressure in the automated OxiTop respirometer flask. Evolved carbon dioxide was absorbed in sodium hydroxide. The amount of oxygen taken up by the test item (corrected for uptake by blank inoculum, run in parallel) was expressed as a percentage of chemical oxygen demand (COD).

The endogenous activity of the inoculum was checked running parallel blanks with inoculum but without test item. A reference compound (sodium benzoate) was run in parallel to check the operation of the procedures.

A toxicity control (test item and reference compound mixed) was run in parallel, to ensure that the chosen concentration of the test item was not inhibitory to microorganisms.

Degradation was followed by the determination of oxygen uptake and measurements were taken at frequent intervals to allow the identification of the beginning and end of biodegradation.

The test lasted for 28 days.

Because of the nature of biodegradation and of the mixed bacterial populations used as inoculum, the determinations of the biodegradation of the test item and the reference compound were carried out in duplicate and those of the inoculum blank in triplicate.

The oxygen uptake was calculated from the readings taken at regular and frequent intervals, using the method given by the manufacturer of the equipment. At the end of incubation, the pH was measured in the flasks.

Start of the incubation period : 2010-06-09

End of the incubation period : 2010-07-07

8. MATERIALS AND METHODS

8.1 Sample description

Test item : PES Vorstufe 2342

Chemical name : Castor Oil, reaction product with

Soybean Oil

Study No.: 2010/0087/14

CAS name : -
CAS number : -
EC/NLP number : -
Sample provided by : -
Empirical formula : --

Molecular mass : -- g/mol

Structural formula : --

Batch number : LB06603520

Charge --

Sample number : 1199

Date of receipt : 2010-04-27 Expiry date : 2010-10-22

Purity : 100 % (according to data of the sponsor)

Water solubility : --

Vapour pressure : ca 4 hPa at 20 °C

8.2 Test organisms

Type : mixed population of aquatic microorganisms

(activated sludge)

Origin : aeration tank of a wastewater plant treating

predominantly domestic sewage

(Wupper area water authority, STP Odenthal)

Study No.: 2010/0087/14

Date of collection : 2010-06-08

Concentration of

inoculum : 30 mg/L suspended solids

8.3 Pre-treatment of the inoculum

 The sludge was washed twice by adding mineral medium and centrifuging for 10 min at 2000 rpm and 20 °C and decanting off the supernatant.

- 1.049 g of the wet sludge was dried in order to determine the wet weight / dry weight ratio of the sludge and to prepare a stock suspension (activated sludge) of 3 g dw/L.
- The calculated amount of sludge, needed to achieve 300 mL of this stock suspension, was dissolved in mineral medium and then filled up to a defined end volume.
 - Before use, the inoculum was stored for one day at room temperature under continuous shaking with aeration.

8.4 Mineral medium

The mineral medium was prepared from stock solutions of mineral components described as follows.

1. Mineral salt solution : Potassium phosphate, monobasic (KH₂PO₄) 8.5 g

*di-*Potassium hydrogen phosphate (K₂HPO₄) 21.75 g

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di-Sodium hydrogen phosphate dihydrate

 $\begin{array}{ll} \text{(Na$_2$HPO$_4$ \cdot 2 H$_2$O)} & 33.4 \text{ g} \\ \text{Ammonium chloride (NH$_4$Cl)} & 0.5 \text{ g} \\ \end{array}$

The components were dissolved in deionised water and

filled up to 1 litre.

The pH of the solution was adjusted to 7.4.

2. Magnesium sulphate

solution : Magnesium sulphate heptahydrate

 $(MgSO_4 \cdot 7 H_2O)$ 22.5 g

The substance was dissolved in deionised water and

filled up to 1 litre.

3. Calcium chloride

solution : Calcium chloride dihydrate

 $(CaCl_2 \cdot 2 H_2O)$ 36.4 g

The substance was dissolved in deionised water and

filled up to 1 litre.

4. Iron (III) chloride

solution : Iron (III) chloride hexahydrate

 $(FeCl_3 \cdot 6 H_2O)$ 0.25 g

The substance was dissolved in deionised water and

filled up to 1 litre.

To prepare the mineral medium 10 mL of the mineral salt solution were mixed with 800 mL deionised water. Then 1 mL each of the magnesium sulphate-, calcium chloride- and the iron (III) chloride solution were added and filled up to 1 litre with deionised water.

8.5 Pre-treatment of the test item

25 mg of the test item were weighed out and added to the test flasks, filled with 200 mL of mineral medium. Afterwards the volume was made up to 250 mL with mineral medium containing the inoculum to give a test concentration of 100 mg test item/L.

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8.6 Pre-treatment of the reference compound

Reference compound name : sodium benzoate (Sigma-Aldrich)

Purity : 99.7 %

Batch-no. : 1438955

25 mg of the reference compound were weighed out and added to the test flasks, filled with 200 mL of mineral medium. Afterwards the volume was made up to 250 mL with mineral medium containing the inoculum to give a test concentration of 100 mg reference compound /L.

8.7 Pre-treatment of the toxicity control

25 mg of the test item and 25 mg of the reference compound were weighed out and added to the test flasks, filled with 200 mL of mineral medium. Afterwards the flask volume was made up to 250 mL with mineral medium containing the inoculum to give a test concentration of 100 mg test item and reference compound/L.

8.8 Exposure conditions

Test volume : 250 mL

Test apparatus : OxiTopControl System (WTW)

Mixing : 1 magnetic stirrer per test vessel

Incubation time : 28 days

Incubation temperature : 22 ± 1 °C

8.9 Chemical analysis

COD

Method : Determination of Chemical Oxygen

Demand of solids and substances which

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are not water-soluble (COD).

Calibration Standard : Potassium hydrogen phthalate

Test apparatus : Titrator / Metrohm 730 / 759

8.10 Applied SOPs and methods

00090 Version 1 Manometric Respirometry Test

Determination of Chemical Oxygen

2011-0619601-07D Demand of solids and substances which

are not water soluble

Deviations : none

9. RESULTS

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Table 1: BOD values (mg O₂/L) after n days

	test ite	em	blank		blank mean	reference compound		toxicity control	
flask no.	18	19	1	2	3		4	5	20
code*	-1	- 0	h 1	۲.	L O	la sea	1	?	14
time [d]	a1	a2	b1	b2	b3	bm	r1	r2	t1
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	26.6	21.0	8.4	5.6	7.0	7.0	35.0	35.0	44.7
2	50.3	43.3	11.2	9.8	9.8	10.3	82.5	78.3	81.1
3	72.7	65.7	18.2	16.8	16.8	17.3	99.3	99.3	102.0
4	90.9	85.3	19.6	18.2	18.2	18.7	109.0	109.0	124.0
5	106.0	109.0	21.0	19.6	21.0	20.5	119.0	123.0	145.0
6	122.0	127.0	22.4	19.6	22.4	21.5	131.0	133.0	164.0
7	131.0	143.0	23.8	21.0	23.8	22.9	140.0	137.0	179.0
8	141.0	154.0	23.8	21.0	25.2	23.3	144.0	141.0	192.0
9	152.0	164.0	22.4	19.6	23.8	21.9	147.0	143.0	204.0
10	161.0	175.0	23.8	22.4	26.6	24.3	150.0	147.0	213.0
11	169.0	182.0	25.2	22.4	28.0	25.2	154.0	151.0	222.0
12	176.0	187.0	23.8	21.0	28.0	24.3	155.0	152.0	232.0
13	182.0	194.0	26.6	22.4	29.4	26.1	158.0	157.0	242.0
14	187.0	201.0	25.2	25.2	30.8	27.1	159.0	158.0	252.0
15	194.0	207.0	25.2	22.4	30.8	26.1	162.0	161.0	263.0
16	201.0	217.0	23.8	23.8	30.8	26.1	164.0	164.0	277.0
17	210.0	222.0	26.6	23.8	32.2	27.5	168.0	166.0	284.0
18	215.0	228.0	25.2	25.2	32.2	27.5	169.0	168.0	288.0
19	221.0	234.0	26.6	23.8	33.6	28.0	171.0	171.0	288.0
20	225.0	239.0	25.2	26.6	35.0	28.9	172.0	172.0	288.0
21	231.0	242.0	26.6	25.2	35.0	28.9	172.0	175.0	288.0
22	235.0	246.0	26.6	26.6	35.0	29.4	173.0	178.0	288.0
23	239.0	250.0	29.4	26.6	39.2	31.7	176.0	182.0	288.0
24	243.0	253.0	28.0	25.2	37.8	30.3	176.0	185.0	288.0
25	246.0	256.0	28.0	26.6	39.2	31.3	178.0	186.0	287.0
26	249.0	260.0	26.6	28.0	39.2	31.3	178.0	187.0	287.0
27	253.0	263.0	29.4	28.0	40.6	32.7	179.0	190.0	287.0
28	253.0	264.0	29.4	28.0	41.9	33.1	179.0	192.0	287.0

Comments: *Abbreviations according to test guideline.

Table 2: pH-values after 28 days

flask no.	pH-value
1	7.5
2	7.5
3	7.5
4	8.0
5	7.9
18	7.6
19	7.6
20	8.1

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Table 3: Degradation of test item

Test concentration : 100 mg/L
Chemical oxygen demand : 3.110 mg O₂/mg

(analytically determined)

	blank-corrected BOD [mg O ₂ /L]		% degr	adation	% degradation mean
time [d]	a1	a2	a1	a2	mean
0	0.0	0.0	0	0	0
1	19.6	14.0	6	5	5
2	40.0	33.0	13	11	12
3	55.4	48.4	18	16	17
4	72.2	66.6	23	21	22
5	85.5	88.5	27	28	28
6	100.5	105.5	32	34	33
7	108.1	120.1	35	39	37
8	117.7	130.7	38	42	40
9	130.1	142.1	42	46	44
10	136.7	150.7	44	48	46
11	143.8	156.8	46	50	48
12	151.7	162.7	49	52	51
13	155.9	167.9	50	54	52
14	159.9	173.9	51	56	54
15	167.9	180.9	54	58	56
16	174.9	190.9	56	61	59
17	182.5	194.5	59	63	61
18	187.5	200.5	60	64	62
19	193.0	206.0	62	66	64
20	196.1	210.1	63	68	65
21	202.1	213.1	65	69	67
22	205.6	216.6	66	70	68
23	207.3	218.3	67	70	68
24	212.7	222.7	68	72	70
25	214.7	224.7	69	72	71
26	217.7	228.7	70	74	72
27	220.3	230.3	71	74	72
28	219.9	230.9	71	74	72

Comments: none

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Table 4: Degradation of reference compound

Test concentration

100 mg/L 1.665 mg O₂/mg rest concentration
Theoretical oxygen demand

	blank-corrected BOD [mg O ₂ /L]		% degradation		% degradation mean
time [d]	r1	r2	r1	r2	mean
0	0.0	0.0	0	0	0
1	28.0	28.0	17	17	17
2	72.2	68.0	43	41	42
3	82.0	82.0	49	49	49
4	90.3	90.3	54	54	54
5	98.5	102.5	59	62	60
6	109.5	111.5	66	67	66
7	117.1	114.1	70	69	69
8	120.7	117.7	72	71	72
9	125.1	121.1	75	73	74
10	125.7	122.7	76	74	75
11	128.8	125.8	77	76	76
12	130.7	127.7	79	77	78
13	131.9	130.9	79	79	79
14	131.9	130.9	79	79	79
15	135.9	134.9	82	81	81
16	137.9	137.9	83	83	83
17	140.5	138.5	84	83	84
18	141.5	140.5	85	84	85
19	143.0	143.0	86	86	86
20	143.1	143.1	86	86	86
21	143.1	146.1	86	88	87
22	143.6	148.6	86	89	88
23	144.3	150.3	87	90	88
24	145.7	154.7	87	93	90
25	146.7	154.7	88	93	91
26	146.7	155.7	88	94	91
27	146.3	157.3	88	94	91
28	145.9	158.9	88	95	92

Comments: none

Table 5: Toxicity control

Test concentration test item : 100 mg/L
Test concentration reference compound : 100 mg/L

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	blank-corrected BOD [mg O ₂ /L]	% degradation
time [d]	t1	t1
0	0.0	0
1	37.7	8
2	70.8	15
3 4 5	84.7	18
4	105.3	22
	124.5	26
6	142.5	30
7	156.1	33
8	168.7	35
9	182.1	38
10	188.7	40
11	196.8	41
12	207.7	44
13	215.9	45
14	224.9	47
15	236.9	50
16	250.9	53
17	256.5	54
18	260.5	55
19	260.0	54
20	259.1	54
21	259.1	54
22	258.6	54
23	256.3	54
24	257.7	54
25	255.7	54
26	255.7	54
27	254.3	53
28	253.9	53

Comments: The used concentration of the test item is not toxic to bacteria.

Within 28 days, a degradation rate of 72 % was determined for PES Vorstufe 2342, based on COD removal. On the other hand, the pass level for Ready Biodegradability (60% removal of COD) according to OECD 301) was not reached. The 10d-window concept means that the time period between 10 % removal of COD and 60 % removal of COD is 10 days or less.

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PES Vorstufe 2342 is considered to be "Not Readily Biodegradable".

With an overall degradation rate of 72 % within 28 days, and about 50 % COD removal within the 10-d-window, the substance clearly exhibits its potential to become degraded by aerobic bacteria under environmental conditions. The pass levels for ready biodegradability, as defined by OECD 301, have only marginally been failed to reach. It appears that, with more favourable conditions or a slightly elongated exposure period, PES Vorstufe 2342 would be easily degraded to the levels required by OECD 301.

The degradation curves are given in figure 1.

9.1 Comments

All validity criteria of the test method were met:

The reference compound reached the level of \geq 60 % for ready biodegradability within 14 days (table 4).

The toxicity control exhibited degradation rates > 25 % within 14 days (table 5).

At the end of the test, at the plateau, or the end of the 10-d window, biodegradation in parallels with test item did not differ by more than 20 percentage points (table 3).

The oxygen uptake of the inoculum blank was \leq 60 mg/L (table 1).

In cases where the degradation was \leq 60 %, the pH values (table 2) were in between 6.0 and 8.5 at the end of the test (and thus poor degradation was not caused by pH influences).

10. DEGRADATION CURVES

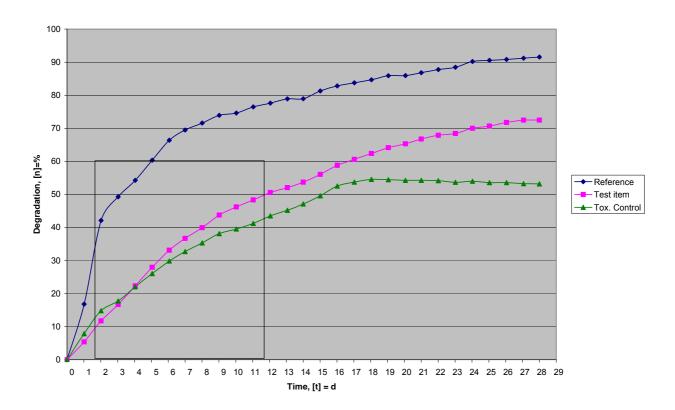


Fig. 1 Degradation curves of PES Vorstufe 2342, toxicity control and reference compound sodium benzoate.